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Please file the following enclosed patent application papers:
Applicant #1: Jeffrey A. Ledbetter Applicant #2: Martha Hayden-Ledbetter Title: DNA Vaccines Encoding Antigen Linked to a Domain that binds CD40
× Fee Transmittal Form
Specification, Claims, and Abstract//Total Pages: 21
Drawings//Total Pages: 7
Declaration//Total Pages 3
a.Newly executed (original or copy) b.Date Signed: 2000 Oct. 13
Nucleotide and/or amino acid sequence submission a.computer readable copy b.paper copy, identical to computer copy
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x Return receipt postcard addressed to applicant #1.
Check for \$380.00 for filing fee for utility patent.
Request under MPEP & 707.07(j): The undersigned, a pro se applicant, respectfully requests that if the Examiner find patentable subject matter disclosed in this application, but feels that Applicant's present claims are not entirely suitable, the Examiner draft one or more allowable claims for applicant.
Very respectfully,
Applicant #1 Signature Applicant #2 Signature Applicant #2 Signature

18798 Ridge Field Rd NW Correspondence Address

Shoreline, WA 98177



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PTO/SB/05 (4/98)

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UTILITY PATENT APPLICATION

Attorney Docket No. First Inventor or Application Identifier Jeffrey Ledbetter DNA Vaccines Encoding Antigen Linked

TRANSMITTAL
(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b)

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Address	18798	Ridgefield	Road N.W.					
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City	USA	THE	State	(206)	546-0473	Fax	(206)	546-6002
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FEE TRANSMITTAL	Co	mplete if Known	_
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for FY 1999	Filing Date		
Patent fees are subject to annual revision.	First Named Inventor	Jeffrey A. Ledbetter	
Small Entity payments <u>must</u> be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12.	Examiner Name		
See 37 C.F.R. §§ 1.27 and 1.28.	Group / Art Unit		_
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SUBMITTED BY	Complete (if applicable)											
Name (PrintlType) Jeffrey A. Ledbetter	Registration No. (Attorney/Agent) Telephone (206) 544	5-0473										

Signature

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STATEMENT CLAIMING SMA (37 CFR 1.9(f) & 1.27(b))IND		Docket Number (Optional)
Applicant, Patentee, or Identifier:	Jeffrey A. Ledbetter	
		
Filed or Issued:		
Title: DNA Vaccines Enco	ding Antigen Linked to a Do	main that Binds CD40
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Jeffrey A. Ledbetter NAMEOFINVENTOR	Martha Hayden-Ledbetter NAMEOFINVENTOR	NAME OF INVENTOR
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Patent Application of Jeffrey A. Ledbetter and Martha Hayden Ledbetter For

TITLE: DNA VACCINES ENCODING ANTIGEN LINKED TO A DOMAIN THAT BINDS CD40

CROSS REFERENCE TO RELATED APPLICATIONS

This application is entitled to the benefit of Provisional Patent Application Ser. # 60/159,690, filed 1999 October 14.

BACKGROUND - FIELD OF INVENTION:

This invention relates to DNA vaccines, specifically to improved DNA vaccines that induce strong antigen-specific humoral and cellular immune responses.

BACKGROUND- DESCRIPTION OF PRIOR ART

DNA immunization, the inoculation of plasmid DNA encoding a microbial or tumor antigen, is a recent addition to vaccine technology (Donnelly J.J. et al, Ann. Rev. Immunol. 15: 617-648, 1997; Letvin N. L., Science 280: 1875-1879, 1998). Both cellular and humoral immune responses occur after DNA vaccination, and protective immunity against microbial challenge is sometimes induced in experimental animals (Ulmer J.B. et al, Vaccine 12: 1541-1544, 1994; Yokoyama M. et al, J. Virol. 69: 2684-2688, 1995; Xiang Z.Q. et al, Virology 199: 132-140, 1994; Sedegah M. et al, Proc. Natl. Acad. Sci. USA 91: 9866-9870, 1994; Montgomery D.L. et al, DNA Cell Biol. 12: 777-783, 1993). T cell responses, including CD8+ cytotoxic T lymphocyte (CTL) and CD4+ T helper cells, can be stimulated by DNA vaccination in response to antigenic peptides presented by class I and class II MHC molecules (Whitton J.L. et al, Vaccine 17: 1612-1619, 1999).

Endogenous protein synthesis allows presentation of foreign antigenic peptides by MHC class I, whereas uptake of soluble protein by APC is required for presentation of peptides by MHC class II. Both arms of the immune response can therefore be induced after DNA vaccination, but the pathways for antigen processing and presentation are distinct for peptides presented by MHC class I or MHC class II. This conclusion is derived from experiments using DNA encoding ubiquitinated protein that is rapidly targeted to intracellular degradation by proteosomes. Ubiquitinated antigen that was degraded so rapidly that intact protein could not leave the cell led to enhanced production of CTL *in vivo*, but completely eliminated antibody production (Rodriguez F. et al, J. Virol. 71: 8497-8503, 1997; Wu Y. and Kipps T.J., J. Immunol. 159: 6037-6043, 1997). Thus a major limitation of DNA vaccines is their inability to induce strong and sustained humoral immune responses. Strategies for optimization of the cellular immune response to DNA vaccines that do not reduce humoral immune responses are needed.

DNA vaccines for HIV-1 have been tested in animal models and found to induce an immune response that provides protection against challenge only when the virulence of the viral isolate is low. In benign challenge models, chimpanzees were protected from live virus exposure by vaccination with plasmid DNA or by subunit antigens or peptides (Boyer J.D. et al, Nat. Med. 3:526-532, 1997; Kennedy R.C., Nat. Med. 3: 501-502, 1997). However, when highly virulent SIV was tested in rhesus macaques, DNA vaccination was not protective and could only achieve a reduction in virus load even when multiple doses of DNA were inoculated through multiple routes (Lu S. et al, J. Virol. 70: 3978-3991, 1996). Therefore, enhancing the immune response to DNA immunization is an important goal of current AIDS vaccine research. Enhancing the immune response to other DNA vaccines is also desirable in order to provide protection when infected with highly virulent organisms or with a high infectious dose, and to provide long lasting protection. Enhancing the immune response to DNA vaccines encoding tumor antigens is also important for maximizing the anti-tumor response.

One strategy that has been tested is to prime with a DNA vaccine followed by boosting with protein antigen. However, this approach requires construction of multiple vaccines for the same infection or disease, and depends upon multiple injections given in a precise order. It would be desirable to induce protective immunity without needing

multiple forms of a vaccine, and without requiring alternating injections of DNA and protein.

Chemical and genetic approaches to enhance the immune response to DNA vaccines have been studied. Chemical adjuvants with some activity include monophosphoryl lipid A (Sasaki S. et al, Infect. Immun. 65: 3520-3528, 1997), saponin QS-21 (Sasaki S et al, J. Virol. 72: 4931-4939, 1998), mannan-coated liposomes (Toda S et al, Immunology 92: 111-117, 1997), and the aminopeptidase inhibitor ubenimex (Sasaki S et al, Clin. Exp. Immunol. 11: 30-36, 1998). Each of these adjuvants modestly enhanced both antibody titers and CTL activity after DNA vaccination in mice. Although the mechanism of action of chemical adjuvants is not fully elucidated, they seem to work by induction of cytokines that amplify responses, by recruitment of macrophages and other lymphoid cells at sites of DNA administration, or by facilitating entry of DNA into host cells (Sasaki S. et al, Anticancer Research 18: 3907-3916, 1998). Several genetic approaches to enhancing responses to DNA vaccines have been tested, including administration of a gene encoding a cytokine (IL2, IL12, GM-CSF, TCA3, MIP-1α) (Chow Y.-H. et al, J. Virol. 71: 169-178,1997; Hwee Lee A. et al, Vaccine 17: 473-479, 1998; Tsuji T. et al, Immunol.158: 4008-4014, 1997; Rodriguez D. et al, Gen. Virol. 80: 217-223, 1999; Tsuji T. et al, Immunology 90: 1-6, 1997; Lu Y. et al, Clin. Exp. Immunol. 115: 335-341,1999) or a costimulatory adhesion receptor (CD86, CD58, CD54) (Tsuji T. et al, Eur. J. Immunol. 27: 782-787, 1997; Kim J.J. et al, J. Clin. Invest. 103: 869-877, 1999; Iwasaki A. et al, J. Immunol. 158: 4591-4601, 1997). Each of these cytokine and adhesion receptor genes increased immune responses to DNA vaccination, with some treatments enhancing CTL generation only, and some enhancing both CTL and antibody production. However, the levels of enhancement of the immune response to DNA vaccination obtained from these approaches are modest and not sustained, so it is important to find additional ways to enhance the immune response to DNA vaccines.

The CD40 receptor must be activated for an effective cellular or humoral immune response after exposure to antigen (Grewal I.S., and Flavell R.A., Annu. Rev. Immunol 16: 111-135, 1998). This conclusion is derived from multiple findings, including the phenotype of patients with hyper IgM (HIGM) syndrome that results from CD154

genetic defects (Aruffo A. et al, Cell 72: 291-300,1993; Fuleihan R. et al, Proc. Natl. Acad. Sci. USA 90: 2170-2173,1993; Korthauer U. et al, Nature 361: 539-541,1993), the phenotype of mice with CD40 or CD154 gene disruption (Grewal I.S. et al., Science 273: 1864-1867,1996; Kawabe T. et al, Immunity 1: 167-178,1994; Renshaw B. et al, J. Exp. Med. 180: 1889-1900,1994; Xu J. et al, Immunity 1: 423-431, 1994), and the effects of actively blocking CD40 in vivo using inhibitory antibodies to CD154 (Durie F.H. et al, Science 261: 1328-1330,1993; Foy T.M. et al, J. Exp. Med. 178: 1567-1575, 1993; Foy T.M. et al, J. Exp. Med. 180: 157-163,1994; Durie F.H. et al, J. Clin. Invest. 94: 1333-1338, 1994; Gerritsse K. et al, Proc. Nat. Acad. Sci. USA 93: 2499-2504, 1996). CD40 is expressed in several cell lineages, including B cells, dendritic cells, monocytes, epithelial cells, and endothelial cells. CD40 transmits signals for each of these cell types that regulates activation and differentiation (Hollenbaugh D. et al, EMBO J. 11: 4313-4321,1992; Kiener P.A. et al, J. Immunol. 155: 4917-4925,1995; Cella M. et al, J. Exp. Med. 184: 747-752,1996; Galy A.H., and Spits H., J. Immunol. 152: 775-782,1992; Clark E.A., and Ledbetter J.A., Proc. Natl. Acad. Sci. USA 83: 4494-4498, 1986). CD40 is activated by crosslinking during cell to cell contact with cells expressing CD40 ligand (CD154), primarily T cells. While soluble forms of CD154 can stimulate CD40, no attempts have been made to use or modify soluble CD154 to promote immune responses to antigens.

CD40 signals to B cells are required for isotype switching and affinity maturation through somatic mutation (Rousset F. et al, J. Exp. Med. 173: 705-710, 1991). In the absence of CD40 signals, germinal centers, the specialized sites of B cell maturation, are not formed, and B cells are unable to differentiate into IgG producing plasma cells (Foy T.M. et al, J. Exp. Med. 180: 157-163, 1994). Patients with HIGM syndrome are not able to form germinal centers or produce IgG antibodies after antigen challenge, and the same phenotype is seen in knockout mice where CD40 or CD154 is not expressed. The CD40 signal has been shown *in vitro* to promote survival of surface Ig-activated B cells, and to interact with signals from cytokines to induce immunoglobulin isotype switching to IgG, IgA, and IgE production (Holder M.J. et al, Eur. J. Immunol 23: 2368-2371,1993; Jabara H.H. et al, J. Exp. Med. 177: 925-935,1990; Grabstein K.H. et al, J. Immunol. 150: 3141-3147, 1993). In addition, HIGM syndrome patients and CD154 knockout mice have impaired lymphocyte proliferation in response to diphtheria toxoid,

tetanus, and *Candida*, showing that the CD40 signal is required for T cell priming to protein antigens (Grewal I.S., and Flavell R.A., Annu. Rev. Immunol 16: 111-135, 1998; Toes R.E.M. et al, Sem. Immun. 10: 443-448,1998; Grewal I.S. et al, Nature 378: 617-620,1995; Ameratunga R. et al, J. Pediatr. 131: 147-150,1997; Subauste C.S. et al, J. Immunol. 162: 6690-6700, 1999). Expression of CD154 *in vivo* to enhance immune responses utilized only the cell surface form of the molecule and resulted in significant toxicity in experimental animals, including induction of lethal autoimmune disease and T cell malignancies (Roskrow M.A et al, Leukemia Research 23: 549-557, 1999; Brown M.P. et al, Nature Medicine 4: 1253-1260, 1998).

In neonates, insufficient stimulation of CD40 due to low levels of expression of CD154 by activated T cells has been identified as a factor in the inability of infants to produce IgG antibodies towards bacterial antigens (Nonoyama S. et al, J. Clin. Invest. 95: 66-75, 1995; Fuleihan R. et al, Eur. J. Immunol. 24: 1925-1928, 1994; Brugnoni D. et al, Eur. J. Immunol. 24: 1919-1924, 1994). This suggests that CD40 signals are not ubiquitous and that highly restricted expression of CD154 may limit the extent of CD40 signaling and thus the magnitude and quality of an immune response. Direct evidence in support of this idea comes from a recent study where a modest increase (1.1-2 fold) in expression of cell surface CD154 in the thymus of mice resulted in a > 10 fold increase in the antigen-specific antibody response (Prez-Melgosa M. et al, J. Immunol. 163: 1123-1127, 1999). Some evidence suggests that CD40 stimulation may be deficient in HIV-1 infected individuals, since HIV gp120 suppressed the expression of CD154 by activated T cells in vitro, and production of IL12 is defective in HIV-1 positive individuals (Chirmule N. et al, J. Immunol. 155: 917-924, 1995; Taoufik Y. et al, Blood 89: 2842-2848, 1997; Yoo J. et al, J. Immunol. 157: 1313-1320, 1996; Ito M. et al, AIDS Res. Hum. Retroviruses 14: 845-849, 1998; Benyoucef S. et al, J. Med. Virol. 55: 209-214, 1998). In addition, CD40 stimulation of dendritic cells infected with HIV-1 was found to suppress virus replication, suggesting that transmission of HIV-1 from infected dendritic cells during antigen presentation could be blocked by CD40 signals (McDyer J.F. et al, J. Immunol. 162: 3711-3717, 1999). However, a method for stimulation of CD40 on cells actively presenting antigen to T cells while avoiding toxicity from unregulated CD40 stimulation is needed.

CD40 signals to dendritic cells or B cells causes their differentiation from an antigen uptake function to an antigen processing and presentation function (Sallusto D. et al, J. Exp. Med. 182: 389-400, 1995; Cella M. et al, J. Exp. Med. 184: 747-752, 1996; Faassen A.E. et al, Eur. J. Immunol. 25: 3249-3255, 1995). This shift is accompanied by reduction of the MHC class II intracellular compartment, increased expression of MHC class II on the cell surface, secretion of the Th1 regulatory cytokine IL12 and increased expression of CD86 and CD80. After CD40 activation, dendritic cells and B cells are able to more efficiently present antigen and give a critical costimulatory signal through CD28. The production of IL12 leads to enhanced secretion of IFNγ by T cells and suppression of Th2 cytokine production. The CD40 signal is therefore an important mediator of Th1 cellular immunity and CTL induction. However, selective stimulation of CD40 during antigen presentation is needed to enhance immune responses to vaccination.

In addition to B cells and dendritic cells, CD40 is functionally active on other APC's such as monocytes, where CD40 signals prevent cell death from apoptosis and induce expression of adhesion molecules and production of inflammatory cytokines TNFα and IL8 (Kiener P.A. et al., J. Immunol. 155: 4917-4925, 1995). CD40 has also been reported to be expressed and functionally active on thymic epithelial cells (Galy A.H., and Spits H., J. Immunol. 152: 775-782, 1992) and on many kinds of tumor cells, including carcinomas, melanomas, and lymphomas (Ledbetter J.A. et al, In Leucocyte Typing III: White Cell Differentiation Antigens p. 432-435, 1987; Oxford University Press, Oxford, U.K.; Paulie S. et al, Cancer Immunol. Immunother. 20: 23-28, 1985). In contrast to most normal cells where the CD40 signal enhances survival, in many malignant cells CD40 actively promotes death by apoptosis. Therefore CD40 is functionally active in all cell types that express the receptor, and CD40 signals are central to fundamental processes of survival and differentiation. Because of the widespread expression of functional CD40, localized stimulation of CD40 positive cells that present specific antigen to T cells is desirable so that only APC involved in the specific immune response are activated.

Studies in CD154 knockout mice have confirmed the importance of CD40 activation for the antigen specific priming of T cells. CD154 deficient mice have an

enhanced susceptibility to *Leishmania major* and *Toxoplasma gondii* infection, consistent with a central role for CD40 in cellular immunity (Subauste C.S. et al, J. Immunol. 162: 6690-6700, 1999; Campbell K.A. et al, Immunity 4: 283-289, 1996). CTL generation after viral infection in CD154 deficient mice is markedly blunted, and induction of experimental allergic encephalomyelitis (EAE) in response to myelin basic protein does not occur (Grewal I.S. et al, Science 273: 1864-1867, 1996; Grewal I.S. et al, 378: 617-620, 1995). The defect in T cell priming in these models appears to be due to an inability of APC to provide costimulatory signals to T cells (Grewal I.S. et al, Science 273: 1864-1867, 1996; Yang Y. and Wilson J.M., Science 273: 1862-1867, 1996).

Inhibition of CD40 *in vivo* has been studied in mice using a mAb, MR1, that binds and blocks the CD40 ligand, CD154 (Durie F.H. et al, Science 261: 1328-1330, 1993; Foy T.M. et al, J. Exp. Med. 178: 1567-1575, 1993; Foy T.M. et al, J. Exp. Med. 180: 157-163, 1994; Durie F.H. et al, J. Clin. Invest. 94: 1333-1338, 1994; Gerritsse K. et al, Proc. Nat. Acad. Sci. USA 93: 2499-2504, 1996). These experiments demonstrated that anti-CD154 prevents the induction of autoimmune diseases, including EAE after immunization with myelin basic protein, oophritis after immunization with zona pelucida antigen (ZP3), and spontaneous disease in lupus prone mice (Griggs N.D. et al, J. Exp. Med. 183: 801-807, 1996; Daikh D.I. et al, J. Immunol. 159: 3104-3108, 1997). Anti-CD154 was also effective in preventing both chronic and acute graft versus host (GVH) disease and in preventing rejection of heart allografts after transplantation (Larsen C.P. et al, Nature 381: 434-438, 1996). Thus, CD40 signals are required for T cell responses to antigen, and restriction of the CD40 signal with specific inhibitors is an effective method of limiting T cell priming during an immune response.

The CD40 receptor is therefore a proven target for regulation of antigen specific immunity. While biological inhibitors of CD40 have been studied extensively in mice and in nonhuman primates, there is a need for localized stimulation of CD40 on cells that present antigens to T cells in order to improve the effectiveness of vaccines.

Gp160, the product of the HIV-1 env gene, is cleaved in the Golgi complex into gp120 and gp41 proteins that remain associated through noncovalent interactions. Most

neutralizing epitopes of the virus are located on gp120 and gp41, and are expressed by the intact env complex that has been shown to be a trimer (Kwong P.D. et al, Nature 393: 648-659, 1998). Monomeric gp120 can be released from the complex and expose immunodominant epitopes that are non-neutralizing and are located on the internal face of gp120 in the intact trimeric complex (Wyatt R. et al, Nature 393: 705-711, 1998; Broder C.C. et al, PNAS USA 91: 11699-11703, 1994). Thus, stabilization of the env complex is needed for an HIV-1 vaccine in order to preserve conformational epitopes important for neutralization and to mask immunodominant epitopes that are not relevant for neutralization of the env complex.

One attempt to produce a stable, properly folded gp120-gp41 complex was made by altering the cleavage site in gp160 between the gp120 and gp41 domains (Earl P.L. et al, J. Virol. 68: 3015-3026, 1994). By introducing a stop codon before the transmembrane domain of gp41, a soluble molecule composed of gp120 and the extracellular domain of gp41 was produced as a complex that folds properly to bind the CD4 receptor and to express some conformational epitopes. However, this molecule formed dimers and multimers rather than the stable trimers that comprise the native structure of the envelope glycoprotein as revealed in the crystal structure of the gp120 complex.

Three major sites of gp120 have been identified that are involved in cross-neutralization of diverse viral strains (Wyatt R. et al, Nature 393: 705-711, 1998). The V3 domain was found to express linear and conformational epitopes that can be recognized by antibodies that neutralize HIV-1. Although the V3 domain is a variable region, it contains a central portion shared by many HIV-1 isolates, particularly those found in the United States and Europe. The central portion has been called the principle neutralization epitope and is formed from a linear epitope of the amino acid sequence GPGRAF (Broliden P.A. et al, Proc. Natl. Acad. Sci. USA 89: 461-465, 1992; Broliden P.A. et al, Immunol. 73: 371-376, 1991; Javaherian K. et al, Science 250: 1590-1593, 1990; Javaherian K. et al, Proc. Natl. Acad. Sci. USA 86: 6768-6772, 1989). Conformational epitopes of the V3 loop have also been identified that can be recognized by antibodies that are more broadly neutralizing.

The CD4 binding domain of gp120 is another neutralization site for antibodies directed to HIV-1 env. This domain is a nonlinear, conformational site that depends upon proper folding of gp120 (Kang C.-Y. et al, Proc. Natl. Acad. Sci. USA: 6171-6175, 1991; Lasky L.A. et al, Cell 50: 975-985, 1987). Antibodies can recognize distinct portions of the CD4 binding domain, and may have either type-specific or crossneutralization properties (Pinter A. et al, AIDS Res. Hum. Retro. 9: 985-996, 1993). Although monomeric gp120 can retain CD4 binding function, a stable trimeric structure of gp120 is thought to be important for masking immunodominant epitopes that are expressed on the internal face of the intact complex (Wyatt R. et al, Nature 393: 705-711, 1998). A third domain of gp120 involved in virus neutralization is exposed upon binding to CD4, and functions to bind the chemokine coreceptor to allow virus entry into the cell (Rizzuto C.D. et al, Science 280: 1949-1953, 1998). Thus a stable trimer of HIV-1 env is needed to present the major cross-neutralization epitopes and to prevent exposure of internal, immunodominant epitopes that do not induce neutralizing antibodies.

CD154 is a TNF-related, type II membrane protein that forms stable trimers (Mazzei G.J. et al, J. Biol. Chem. 270: 7025-7028, 1995). Soluble fusion proteins of human CD154 have been expressed using murine CD8 at the amino terminal side of the CD154 molecule (Hollenbaugh D. et al, EMBO J. 11: 4313-4321, 1992). Single chain Fv (scFv) molecules have also been constructed using heavy and light chain variable regions cloned from the G28-5 hybridoma that produces antibody specific for human CD40 (Ledbetter J.A. et al, Crit. Rev. Immunol.17: 427-435, 1997). Both CD154 and G28-5 scFv fusion proteins retain functional activity as soluble molecules *in vitro*. However, no use of these molecules to improve the effectiveness of vaccines has been found.

SUMMARY

For vaccines to be effective, they must induce both humoral and cellular immune responses. This invention describes improved vaccines that target antigens to cell surface receptors. DNA vaccines are a recent addition to immunization technology. However, further optimization of DNA vaccines is needed to induce long-lasting

protection against tumor antigens, virulent HIV-1 isolates, and other pathogenic microorganisms. Receptor activation and targeting improves the ability of DNA vaccines to generate strong cellular immunity and high titers of neutralizing antibodies. CD40 is a preferred receptor for targeting and activation. DNA vaccines encoding CD40 ligand (CD154) or a single chain Fv (scFv) specific for CD40, fused with DNA encoding portions of the HIV-1 env protein are preferred embodiments of the invention. A molecule comprising the extracellular domain of HIV-1env gp160 or env gp120 linked to the extracellular domain of CD154 is a stable trimer that improves immune recognition of HIV-1 env cross-neutralization epitopes. After DNA vaccination, the expression of the fusion protein in vivo results in both activation of the CD40 receptor and direction of HIV-1 env antigens into the endocytic pathway of CD40 positive antigen presenting cells (APC). Internalization of env antigens after binding the CD40 receptor enhances presentation of peptides by MHC molecules. Activation of the CD40 receptor promotes B cell and APC maturation leading to effective antibody production and generation of CD4+ helper T cell and CD8+ CTL activity. The combination of CD40 activation, stabilization of the HIV-1 gp160 or gp120 env trimer, and enhanced presentation of antigenic peptides by MHC molecules thus improves immune responses to HIV-1 antigens. Protein molecules of the invention can be injected directly into mammals or encoded by DNA vaccines.

DRAWINGS

Figure 1.

Schematic representation of fusion proteins that target antigen to cell surface receptors expressed by antigen presenting cells.

- A. A fusion protein expressed from a cDNA construct that encodes an antigen domain attached with a linker to a receptor targeting domain. The antigen domain may be attached to the amino terminus of the receptor targeting domain as shown, or may be attached to the carboxy terminus of the receptor targeting domain.
- B. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to the amino terminus of the CD154 extracellular domain.

- C. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to the amino terminus of a single chain Fv specific for CD40.
- D. A fusion protein expressed from a cDNA construct as in C, except that the scFv that binds CD40 is oriented with the light chain variable region (V_L) attached to the carboxy-terminus of the heavy chain variable region (V_H) .
- E. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to a camelid variable region (V_{HH}) that binds CD40.
- F. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to a peptide that binds CD40.

Figure 2.

A. Sequence of two cDNAs encoding HIV gp120-V3 loop/CD154 long form extracellular domain fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV V3 loop from gp120 with a (ProAspPro) linker (SEQUENCE ID NO.: 17 [DNA] OR SEQUENCE ID NO.: 25 [FUSION PROTEIN]) or a (Gly₄Ser)₃ linker (SEQ. ID NO.: 16 [DNA] OR SEQ. ID NO.:24 [FUSION PROTEIN]) fused to the CD154 extracellular domain encoded between amino acids 48 (Arg)-261(Leu), with an additional (Glu) residue at the carboxyl end of the protein, not present in wild type CD154. The sequence of the fusion protein is indicated using the three-letter amino acid code convention, above each codon of the open reading frame. Relevant restriction sites are indicated on the drawing and the nucleotides encoding sites at domain fusion junctions are displayed in boldface type, while the first codon of each fused domain is indicated in underlined, italicized type. The protein domains are labeled above the relevant position in the sequence. The nucleotide number is indicated in the left margin with a designation for the PDP linker form or the G4S linker form.

B. Sequence of two cDNAs encoding HIV V3 loop-CD154 short form extracellular domain fusion proteins.

The two HIV V3 loop constructs with alternate linkers, either (ProAspPro) (SEQUENCE ID NO.:19 [DNA] OR SEQUENCE ID NO.: 27 [FUSION PROTEIN]) or (Gly₄Ser)₃ (SEQUENCE ID NO.: 18 [DNA] OR SEQUENCE ID NO.: 26 [FUSION PROTEIN])

were also fused to the short form of the CD154 extracellular domain encoded from amino acids 108 (Glu)-261 (Leu) plus an extra glutamic acid residue at the carboxy terminus, not encoded by wild type CD154. All sequences are labeled as described for Figure 2A.

Figure 3.

A. Sequence of two HIV gp120env-CD154 long form extracellular domain cDNA and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 13 [DNA] OR SEQ. ID NO.: 21 [FUSION PROTEIN]) or a (Gly₄Ser)₃ linker (SEQ. ID NO.: 12 [DNA] OR SEQ. ID NO.: 20 [FUSION PROTEIN]) fused to the CD154 extracellular domain (Long Form) encoded between amino acids 48 (Arg)-261(Leu) + (Glu). All sequences are labeled as described for Figure 2A.

B. Sequence of two HIV gp120env-CD154 short form extracellular domain cDNAs and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 15 [DNA] or SEQ. ID NO.: 23 [fusion protein]) or a (Gly4Ser)3 linker (SEQ. ID NO.: 14 [DNA] or SEQ. ID NO.: 22 [fusion protein]) fused to the short form of the CD154 extracellular domain encoded between amino acids 108 (Glu)-261 (Leu) + (Glu).. All sequences are labeled as described for Figure 2A.

DESCRIPTION

This invention relates to improved vaccines comprising one or more antigens attached to a domain that targets at least one cell surface receptor. The vaccine may be delivered either as a protein, as a DNA plasmid, or by a viral vector. The expression of the DNA after injection of the plasmid or viral vector *in vivo* results in the secretion of the antigen(s) attached to a targeting domain, directing the antigen(s) to a cell surface receptor. Receptor-mediated internalization of the antigen into the endocytic compartment of cells that express the receptor enhances the presentation of antigenic peptides by MHC class II molecules that circulate through this compartment.

Presentation of antigenic peptides by MHC class I molecules is mediated by the cells expressing the DNA vaccine, and is enhanced in cells that internalize the antigentargeting domain fusion protein by movement of the fusion protein from the endocytic compartment into the cytoplasm. The activation of antigen-specific CD4+ T cells and CD8+ T cells is increased, resulting in better humoral and cellular immune responses.

The preferred receptor(s) chosen for antigen targeting are those expressed by antigen presenting cells (APC), such as dendritic cells. Desirable receptors for targeting include but are not limited to CD80, CD86, CD83, CD40, CD32, CD64, Flt3, Dec 205, and ICOS ligand. The CD40 receptor is a preferred receptor for antigen targeting, since signals from CD40 regulate activation and differentiation of APC. Fusion proteins of antigen and CD154 (CD40 ligand) combine the functions of antigen targeting and activation of APC by simultaneous delivery of CD40 signals.

The preferred antigen(s) for receptor targeting are HIV-1 and HIV-2 viral antigens, since vaccines have not been effective in protecting against virulent viral isolates. Attachment of HIV-1 gp160 or gp120 extracellular domain to CD154 extracellular domain stabilizes the trimeric structure of HIV-1 env. However, the invention is not limited to HIV env antigens, since improved immune responses to vaccines are needed to provide long-lasting protection against infection with high doses of pathogenic microorganisms or against tumors.

Thus the structure of the invention's main embodiment is a DNA plasmid encoding the extracellular domain of HIV-1 env gp160 attached to the CD154 extracellular domain.

The fusion protein expressed from this DNA plasmid a) stabilizes the trimeric structure of HIV-1 env, b) directs the HIV-1 antigen into the MHC class II compartment of CD40 positive cells, and c) selectively activates the CD40 receptor to increase APC functional activity.

The main embodiment of the invention encodes a stable trimer that expresses the major cross-neutralization epitopes of HIV-1 env while masking the internal env

epitopes that are not involved in virus neutralization. Antigenic peptides of HIV env are presented by MHC class I molecules by cells that express the DNA, while antigenic peptides of HIV env are presented by MHC class II molecules in CD40 positive cells that internalize the trimeric antigen-CD154 fusion protein. Activation of the CD40 receptor on cells bound by the antigen-CD154 fusion protein increases the specific immune response due to increased production of IL12 and increased expression of costimulatory molecules CD80 and CD86.

OPERATION

An improved DNA vaccine for AIDS comprising the extracellular domain of HIV-1 gp160, HIV-1 gp120, or a subdomain of these antigens fused to the extracellular domain of CD154 is described. Alternative embodiments of the invention use a smaller portion of the CD154 molecule composed of an 18 kDa subunit from Glu-108 to Leu-261 (Mazzei G.J. et al, J. Biol. Chem. 270: 7025-7028, 1995). The extracellular domain of gp160 can also be shortened by removing the gp41 domain, removing the V1 and V2 domains, or mutating the glycosylation sites without damaging the conformational structure of the HIV-1 envelope (Kwong P.D. et al, Nature 393: 648-659, 1998). These changes could further improve the activity of the vaccine, since the V1 and V2 loops, and the carbohydrate structures are thought to be exposed, clade specific epitopes that prevent or dilute the immune response to important cross-neutralization epitopes for diverse clades of HIV-1. Linkers between gpl 60 and CD154 can also be used. Thus, alternative embodiments of the invention minimize the CD154 domain, remove gp41, V1, V2, or glycosylation sites of gp160. This invention also envisions DNA vaccines comprising other HIV-1 antigens and antigens from alternative isolates of HIV-1, fused to the extracellular domain of CD154.

Delivery of antigen(s) to the CD40 receptor may use anti-CD40 scFv instead of CD154. Single antibody variable regions (V_{HH}) or peptides that bind CD40 are also included in the scope of the invention.

Antigen targeting to receptors is not limited to the CD40 receptor. Alternative receptors preferred for targeting include CD80, CD86, Dec205, ICOS ligand, Flt 3, Fc

receptors, and CD83. All cell surface receptors are envisioned by this invention. Receptors may be targeted by ligands, scFv molecules, single variable regions or peptides. Additional methods of attachment of antigen(s) to receptor targeting domains are envisioned, including chemical linkages of subunits, disulfide bonds, or noncovalent attachments such as leucine zipper motifs and the like. The invention contemplates injection of protein, injection of DNA plasmids, or viral vectors encoding the molecules comprising one or more antigens linked to a receptor-binding domain.

Antigens targeted to cell surface receptors are not limited to HIV gp160 antigens. Other antigens, including tumor antigens, parasite antigens, bacterial antigens, and viral antigens are included in the scope of the invention.

The invention also envisions delivery of antigens to cell surface receptors in order to induce antigen-specific tolerance or nonresponsiveness. For this application, an autoantigen would be chosen and the vaccine would be used to treat autoimmune disease.

The invention also envisions antigen(s) that are natural components of the body, such as tumor-associated antigens, where an immune response to the antigen(s) breaks tolerance to the antigen, resulting in a change in immune homeostasis.

The following examples describe particular embodiments of the invention but are not meant to limit its scope.

EXAMPLE 1

A preferred embodiment of the DNA vaccine includes an amino-terminal secretory signal peptide sequence upstream and adjacent to a cDNA sequence cassette encoding the desired antigen. This molecule is then fused to the extracellular domain of CD154 or to a portion of the extracellular domain of CD154 which retains the ability to bind CD40, or to an scFv targeted to CD40, to create a fusion protein expression cassette that targets the antigen to the antigen presenting cell through the CD40 receptor as diagrammed in Figure 1. The expression cassette is inserted into an appropriate mammalian expression vector or virus to achieve high level expression of the fusion protein either *in vitro* or *in vivo*.

The leader peptide is encoded on complementary oligonucleotides with a single-stranded HindIII cohesive end at the 5' terminus, and a BglII cohesive end at the 3' terminus. The sense oligonucleotide is designated SEQUENCE ID NO: 1 or HBLPS and the sequence is as follows:

5'agcttgccgccatgctgtatacctctcagctgttaggactacttctgttttggatctcggcttcga-3'.

The antisense oligonucleotide is designated SEQUENCE ID NO: 2 or HBLPAS and the sequence is as follows:

5'gatctcgaagcccgagatccaaaacagaagtagtcctaacagctgagaggtatacagcatggcggca-3'. The two molecules anneal to one another except at the overhanging nucleotides indicated in boldface type. Alternative embodiments could include other secretory signal peptides or localization sequences.

The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin). Two different fusion junctions were designed which resulted in a short or truncated form (form S4) including amino acids 108 (Glu)-261 (Leu) + (Glu),, and a long or complete form (form L2) including amino acids 48 (Arg) - 261 (Leu) + (Glu), of the extracellular domain of CD154. The sense primer which fuses the extracellular domain to the targeted antigen includes a BamHI site for cloning that introduces the peptide sequence PDP or (ProAspPro) at the fusion junction and can also encode a linker peptide such as (Gly₄Ser)₃ to separate the antigen from the extracellular domain. The oligonucleotide primers used in amplifying the short form (S4) of the CD154 extracellular domain encoding amino acids 108 (Glu)-261 (Leu) + (Glu) are as follows:

The sense primer is designated SEQUENCE ID NO: 3 or CD154BAM108 and encodes a 34 mer with the following sequence: 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3', while the antisense primer is designated SEQUENCE ID NO: 4 or CD154XBA and encodes a 44 mer with the following sequence: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

The oligonucleotide primers used in amplifying the long form (L2) of the CD154 extracellular domain encoding amino acids 48 (Arg)-261 (Leu) + (Glu), are as follows: The sense primer is identified as SEQUENCE ID NO: 5 or CD154 BAM48 and encodes a 35 mer with the following sequence: 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-

3', while the antisense primer is also SEQUENCE ID NO: 4 or CD154XBA encoding the 44 mer: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

A variety of different antigens can be encoded on cDNA cassettes to be inserted between the leader peptide cassette and the CD40 targeted domain (such as a truncated or complete CD154 extracellular domain or a CD40 specific scFv). In a preferred embodiment of the invention, the cDNA antigen encoded by the vaccine is the HIV-1 gp 120 or a fragment of this antigen, such as the V3 loop. The primer sets used to amplify the complete gp120 domain include the sense primer SEQUENCE ID NO: 6 or GP120Bgl2f 5'-gga tat tga tga gat cta gtg cta cag-3' and one of two antisense primers encoding different linkers. Either the antisense primer encoding the ProAspPro linker, identified as SEQUENCE ID NO: 7 or GP120PDPr 5'-gaa cac age tee tat tgg ate egg tet ttt ttc tct ttg cac-3' or the antisense primer encoding the (Gly₄Ser)₃ linker, identified as SEQUENCE ID NO: 8 or GP120G4Sr 5'-cct gca tgg atc cga tcc gcc acc tcc aga acc tcc ace tee tga ace gee tee eee tet ttt tte tet ttg eae tgt tet tet ett tge-3' were used to amplify the gp120 domain with the desired linker attached. PV75Kgp160(89.6) DNA was used as template in PCR reactions. Alternatively, other isolates or sequence variants of gp120 or gp160 are available and can be substituted to create novel fusion cassettes. PCR amplification reactions were performed using cloned plasmid DNA as template (approximately 45 ng), 3 mM MgCl₂, 0,3 MM dNTPs, 1/10 volume 10X reaction buffer supplied by the manufacturer, 10 pmol sense primer, 10 pmol antisense primer, and 2.5 units TAO polymerase (Takara Pharmaceuticals) in a total reaction volume of 50 μl. The amplification profile included an initial 4 minute 94°C denaturation, followed by a 30 cycle program of 50°C annealing for 30 seconds, 72°C extension for 30 seconds, and 94°C denaturation for 30 seconds. PCR fragments were purified by ethanol precipitation, resuspended in 30 µl ddH₂O and 10 µl was digested with BglII (Roche) restriction endonuclease in a 20 µl reaction volume at 37°C for 3 hours. Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN, San Diego, CA), and ligated along with the annealed leader peptide oligonucleotides to HindIII-BamHI digested expression vector already containing the CD154 extracellular domain as a BamHI-XbaI fragment. Recombinant clones were screened for the correct orientation and presence of inserts, and the resulting positive clones were verified by DNA sequencing using an ABI 310 sequence analyzer and the ABI Prism Dye Terminator Reaction Chemistry. The final fusion cassette encodes the

synthetic leader peptide fused to the HIV gp120 domain with either a (ProAspPro) linker or a (Gly₄Ser)₃ linker, and then to the CD154 extracellular domain long (Figure 3A) or short (Figure 3B) form to create the embodiments of example 1.

EXAMPLE 2

In an alternative preferred embodiment, the V1 and V2 domains of gp120 are removed and only the V3 loop domain from HIV gp 120 is encoded on a BglII-BamHI fragment and fused to the signal peptide and the CD154 extracellular domain to create the vaccine, as illustrated in Figure 2A and B. This antigen domain is separated from the CD154 short (Figure 2B) or long extracellular domain (Figure 2A) by a peptide linker encoding the amino acids (ProAspPro), or a longer peptide linker encoding the amino acids (Gly₄Ser)₃.

The V3 loop was PCR amplified from pV75 (gp 89.6), a plasmid containing HIV gp120 from isolate LAV, using the following primer set:

The antisense primer encoding a ProAspPro linker is SEQUENCE ID NO: 9 or V3PDPr 5'-gtt att cca tgg atc cgg act aat ctt aca atg tgc ttg-3'

The sense primer fusing the antigen to the signal peptide is SEQUENCE ID NO: 10 or V3Bgl2f

5'-gta cag cta aat aga tct gta gta att aat tg-3'

The antisense primer encoding a (Gly₄Ser)₃ linker is SEQUENCE ID NO: 11 or V3G4Sr 5'-ggt gca tgg atc cga acc tcc acc gcc aga tcc acc gcc tcc tga ggc acc gcc acc act aat gtt aca atg tgc ttg ttg tct tat atc tcc-3'.

Amplification, digestion, purification, and ligation conditions were identical to those described above for the full-length gp120 domain. The final fusion cassettes encode the HIV gp120-V3 loop with either a (ProAspPro) linker or a (Gly₄Ser)₃ linker fused to either the CD154 extracellular domain as diagrammed in Figure 2A for the long form, and Figure 2B for the short form of the CD40 binding domain.

Other antigens and linkers can be substituted to create alternative vaccines by construction of the appropriate cDNA cassettes encoding the desired domains and attaching them to the CD154 extracellular domain. Because of the high degree of sequence variation among HIV isolates, alternative sequences might be incorporated as needed to target particular clades. Other viral antigens such as HIV tat or their

subdomains can be substituted for the HIV domains described here. Similarly, an alternate APC targeted domain can be substituted for the CD40 binding domain, such as a domain which binds to CD80 or CD86, or to ICOS ligand, or to one of several other cell surface receptors expressed on antigen presenting cells. Surface receptors that internalize readily are preferred over receptors that contain multiple transmembrane domains and do not internalize readily such as G-protein coupled chemokine receptors.

CLAIMS: We claim:

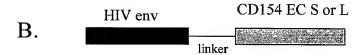
- 1. A vaccine comprising one or more antigens linked to a domain that binds at least one receptor.
- 2. A vaccine of claim 1 where said receptor is CD40.
- 3. A vaccine of claim 1 where said domain is CD154 or a portion of CD154.
- 4. A vaccine of claim 1 where said domain is a single chain Fv that binds CD40.
- 5. A vaccine of claim 1 where said domain binds to one or more receptors selected from the group consisting of CD80, CD86, CD32, CD64, CD83, ICOS ligand, Flt3, CD10, CD11, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD37, CD38, CD39, CD43, CD56, CD58, CD72, CD75, CD76, CD77, CD78, and cytokine/growth factor receptors.
- 6. A vaccine of claim 1 where said antigen is HIV-1 gp160 or a portion of HIV-1 gp160.
- 7. A vaccine of claim 1 where said antigen is a tumor antigen or a microbial antigen.
- 8. A DNA expression plasmid encoding a vaccine comprising one or more antigens linked to a domain that binds at least one receptor.
- A DNA expression plasmid of claim 8 encoding a vaccine where said receptor is CD40.
- 10. A DNA expression plasmid of claim 8 encoding a vaccine where said domain is CD154 or a portion of CD154.
- 11. A DNA expression plasmid of claim 8 encoding a vaccine where said domain is a single chain Fv that binds CD40.
- 12. A DNA expression plasmid of claim 8 encoding a vaccine where said domain binds to one or more antigens selected from the group consisting of CD80, CD86, CD32, CD64, CD83, ICOS ligand, Flt3, CD10, CD11, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD37, CD38, CD39, CD43, CD56, CD58, CD72, CD75, CD76, CD77, CD78, and cytokine/growth factor receptors.
- 13. A DNA expression plasmid of claim 8 encoding a vaccine where said antigen is HIV-1 gp160 or a portion of HIV-1 gp160.
- 14. A DNA expression plasmid of claim 8 encoding a vaccine where said antigen is a tumor antigen or a microbial antigen.

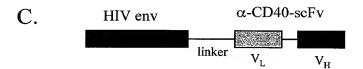
ABSTRACT

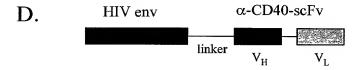
Vaccines that target one or more antigens to a cell surface receptor improve the antigen-specific humoral and cellular immune response. Antigen(s) linked to a domain that binds to a cell surface receptor are internalized, carrying antigen(s) into an intracellular compartment where the antigen(s) are digested into peptides and loaded onto MHC molecules. T cells specific for the peptide antigens are activated, leading to an enhanced immune response. The vaccine may comprise antigen(s) linked to a domain that binds at least one receptor or a DNA plasmid encoding antigen(s) linked to a domain that binds at least one receptor. A preferred embodiment of the invention targets HIV-1 env antigen to the CD40 receptor, resulting in delivery of antigen to CD40 positive cells, and selective activation of the CD40 receptor on cells presenting HIV-1 env antigens to T cells.

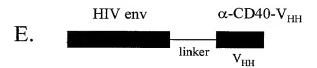
1/7
Figure 1.
Fusion Proteins that Target Antigen to APC

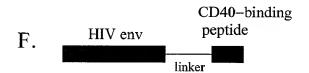












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Figure 2A.
Sequence and translation of two cDNAs encoding HIV gp120 V3 loop-CD154
LONG form extracellular domain fusion proteins.

s	equer	ice an	d trar	ıslatic	n of t		DNA		ding	HIV	gp120	V3 1	oop-C	CD154	1		
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	Hino	III															
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					Met	Leu	Tyr	$\operatorname{\mathtt{Thr}}$	Ser	Gln	Leu	Leu	Gly	Leu	Leu		
1	AAG	CTT	GCC	GCC	ATG	CTG	TAT			CAG	CTG	TTA	GGA	CTA	CTT		
								Bgl1		77 T	· 1 (	20 77					
		m. 1	_	- 1 ·		70.71 -	0				Vgp12				Πp rc		
4.0	Leu	Phe TTT	Trp	TIE	Ser	ALA	Ser	Arg	ner Ter	va⊥ CTA	CTD	7 T.G	ASII	тст			
46		Pro															
91	Arg	CCC	ASII	ASII	ASII		AGA	AGA	AGG	ттА	тСт	ATA	GGA	CCA	GGG		
31	AGA	Ala	Phe	Tyr	Ala	Ara	Ara	Asn	Ile	Ile	Glv	Asp	Ile	Arq	Gln		
136	AL 9	GCA	<u>ф</u> фф	TAT	GCA	AGA	AGA	AAC	ATA	ATA	GGA	GAT	ATA	AGÃ	CAA		
100		His															
181		CAT															
	Pro	AspP:	ro L	inke	r												
	1	BamH:	Γ														
		~~~~		7													
199	1	Asp GAT															
199	CCG	GAI	CCA														
	OR	(Gly	¿Ser)) ₃ Li	nker											amHI	~~~
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199	CCT	GEC	GET.	GGC	TCA	GGA	GGC	GGT	GGA	TCT	GGC	GGT	GGA	GGT	TC G	GAT	CCA
±))																	
			_	ONG					main								
208PD	P			Leu													
250GS				TTG					_		1			_	m¹- ·	т1.	
229PD		Asp	Glu	Arg	Asn	Leu	His	GLu	Asp	Phe	Val	Pne	Met	ьуs	Thr	TTE	
271GS		GAT	GAA	AGG	AAT	CTT	CAT	GAA	GAT	TTT	GTA	TTC	ATG	LOU	ACG	HIA	
274PD		GIn	Arg	Cys	ASN	Thr	GCA	GIU	Arg	TCC	теп	Jet Jet	тта	CTG	Asn AAC	тСт	
316GS	i i	CAG	AGA	. 166	HAC	ACA	GGA	GAA	AUA	100	1 177	100	1 1/1	0.0	1110		

208PDP		Arg													
250 GS	AGA	AGG	TTG	GAC	AAG	ATA	GAA	_		7			-	m¹- ·	т1_
229PDP	Asp	Glu	Arg	Asn	Leu	His	Glu	Asp	Phe	Val	Phe	Met	Lys	rnr	TTE
271GS	GAT	GAA	AGG	AAT	CTT	CAT	GAA	GAT	TTT	GTA	TTC	A'I'G	AAA	ACG	ATA
274PDP	Gln	Arg	Cys	Asn	Thr	Gly	Glu	Arg	Ser	Leu	Ser	Leu	Leu	Asn	Cys
316GS	CAG	AGA	TGC	AAC	ACA	GGA	GAA	AGA	TCC	TTA	TCC	TTA	CTG	AAC	TGT
319PDP	Glu	Glu	Ile	Lys	Ser	Gln	Phe	Glu	Gly	Phe	Val	Lys	Asp	TTe	Met
361GS	GAG	GAG	ATT	AAA	AGC	CAG	TTT	GAA	GGC	TTT	GTG	AAG	GAT	ATA	ATG
364PDP	Leu	Asn	Lys	Glu	Glu	Thr	Lys	Lys	Glu	Asn	Ser	Phe	Glu	Met	GIn
406GS	TTA	AAC	AAA	GAG	GAG	ACG	AAG	AAA	GAA	AAC	AGC	TTT	GAA	ATG	CAA
409PDP	Lys	Gly	Asp	Gln	Asn	Pro	Gln	Ile	Ala	Ala	His	Val	Ile	Ser	Glu
451GS	AAA	GGT	GAT	CAG	TAA	CCT	CAA	ATT	GCG	GCA	CAT	GTC	ATA	AGT	GAG
454PDP	Ala	Ser	Ser	Lys	${ t Thr}$	${ t Thr}$	Ser	Val	Leu	Gln	${\tt Trp}$	Ala	Glu	Lys	Gly
496GS	GCC	AGC	AGT	AAA	ACA	ACA	TCT	GTG	TTA	CAG	TGG	GCT	GAA	AAA	GGA
499PDP	Tvr	Tyr	Thr	Met	Ser	Asn	Asn	Leu	Val	Thr	Leu	Glu	Asn	Gly	Lys
541GS	TAC	TAC	ACC	ATG	AGC	AAC	AAC	TTG	GTA	ACC	CTG	GAA	AAT	GGG	AAA
544PDP	Gln	Leu	Thr	Val	Lys	Arg	Gln	Gly	Leu	Tyr	Tyr	Ile	${ t Tyr}$	Ala	Gln
586GS	CAG	CTG	ACC	GTT	AAA	AGA	CAA	GGA	CTC	TAT	TAT	ATC	TAT	GCC	CAA
589PDP	Val	Thr	Phe	Cys	Ser	Asn	Arg	Glu	Ala	Ser	Ser	Gln	Ala	Pro	Phe
631GS	GTC	ACC	TTC	TGT	TCC	AAT	CGG	GAA	GCT	TCG	AGT	CAA	GCT	CCA	TTT
634 PDP	Ile	Ala	Ser	Leu	Cys	Leu	Lys	Ser	Pro	Gly	Arg	Phe	Glu	Arg	Ile
676GS	ATA	GCC	AGC	CTC	TGC	CTA	AAG	TCC	CCC	GGT	AGA	TTC	GAG	AGA	ATC
679PDP	Leu	Leu	Arg	Ala	Ala	Asn	Thr	His	Ser	Ser	Ala	Lys	${\tt Pro}$	Cys	Gly
721GS	TTA	CTC	AGA	GCT	GCA	AAT	ACC	CAC	AGT	TCC	GCC	AAA	CCT	TGC	GGG
724PDP	Gln	Gln	Ser	Ile	His	Leu	Gly	Gly	Val	Phe	Glu	Leu	Gln	${\tt Pro}$	Gly
766GS	CAA	CAA	TCC	ATT	CAC	TTG	GGA	GGA	GTA	TTT	GAA	TTG	CAA	CCA	GGT
769PDP	Ala	Ser	Val	Phe	Val	Asn	Val	Thr	Asp	Pro	Ser	Gln	Val	Ser	His
811GS	GCT	TCG	GTG	TTT	GTC	AAT	GTG	ACT	GAT	CCA	AGC	CAA	GTG	AGC	CAT
814PDP	Gly	Thr	Gly	Phe	Thr	Ser	Phe	Gly	Leu	Leu	Lys	Leu	Glu	***	***
856GS	GGĈ	ACT	GGC	TTC	ACG	TCC	TTT	GGC	TTA	CTC	AAA	CTC	GAG	TGA	TAA
	Xba														
859PDP	~~~	~~~~													

859PDP ~~~~~ 901GS **TCT AGA**

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Figure 2B.
Sequence and translation of two cDNAs encoding HIV gp120 V3 loop-

	S		nce ar											loop-		
	Hino						_									
	~~~	~~~	-		_		?ept:									
														Leu		
1	AAG	CTT	GCC	GCC	ATG	CTG	TAT							CTA	CTT	
								BglI	II $HI$	[Vgp]	120-T	73 10	goo			
								~~~	~~~	-						
	Leu	Phe	Trp	Ile	Ser	Ala	Ser	Arq	Ser	Val	Val	Ile	Asn	Cys	Thr	
46														TGT		
- 0														Pro		
9_	AGA	CCC	AAC	AAC	AAT	ACA	AGA	AGA	AGG	TTA	TCT	ATA	GGĀ	CCA	GGĞ	
J														Arg		
136														AGA		
136							AGA	AAC	AIA	AIA	GOM	OAI	71171	11011	0111	
			Cys													
181	GCA	CAT	TGT	AAC	ATT	AGT										
	Dwai	A con D	T	inko	<u>~</u>											
	PIO	Baml	ro L: HI ~~~~		_											
	Dro	Asp		Ì												
199		-	CCA													
199	000	GAI	CCA	J												
	OR	(Gly	Ser)	3 Li	nker									I	BamHI	
Glv	Glv	Glv	Glv	Ser	Glv	Glv	Glv	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Pro
199GGT	GGC	GGT	GGC	TCA	GGA	GGC	GGT	GGĀ	TCT	GGĈ	GGT	GGĀ	GGT	TCG	GAT	CCA
133001																
		CD1	54 S	HORT	ext.	race.	11u1.	ar d	omai	n						
208PD	P		Asn													
250GS	-		AAC													
229PD	D								Tle	Ala	Ala	His	Va.1	Ile	Ser	Glu
27100	_														AGT	

	CD154 SHORT	T extracellular domain							
208PDP	Glu Asn Ser	Phe Glu Met	Gln						
250GS		TTT GAA ATG							
229PDP			Gln Ile Ala Ala His Val Ile Ser Glu						
271GS			CAA ATT GCG GCA CAT GTC ATA AGT GAG						
274PDP			Ser Val Leu Gln Trp Ala Glu Lys Gly						
316GS			TCT GTG TTA CAG TGG GCT GAA AAA GGA						
319PDP			Asn Leu Val Thr Leu Glu Asn Gly Lys						
361GS			AAC TTG GTA ACC CTG GAA AAT GGG AAA						
364PDP			Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln						
406GS			CAA GGA CTC TAT TAT ATC TAT GCC CAA						
409PDP			Arg Glu Ala Ser Ser Gln Ala Pro Phe						
451GS			CGG GAA GCT TCG AGT CAA GCT CCA TTT						
454PDP			Lys Ser Pro Gly Arg Phe Glu Arg Ile						
496GS			AAG TCC CCC GGT AGA TTC GAG AGA ATC						
499PDP			Thr His Ser Ser Ala Lys Pro Cys Gly						
5 41GS			ACC CAC AGT TCC GCC AAA CCT TGC GGG						
5 4 4PDP			Gly Gly Val Phe Glu Leu Gln Pro Gly						
586GS			GGA GGA GTA TTT GAA TTG CAA CCA GGT						
589PDP	Ala Ser Val	Phe Val Asn	Val Thr Asp Pro Ser Gln Val Ser His						
631GS			GTG ACT GAT CCA AGC CAA GTG AGC CAT						
634GS	Gly Thr Gly	Phe Thr Ser	Phe Gly Leu Leu Lys Leu Glu *** ***						
676GS		TTC ACG TCC	TTT GGC TTA CTC AAA CTC GAG TGA TAA						
	XbaI								
	~~~~~								
679PDP	Ser Arg								
721GS	TCT AGA								

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Figure 3A.
Sequence and translation of two cDNAs encoding HIV gp120-CD154 LONG form extracellular domain fusion proteins.

	Hind	lIII													
	~~~~	~~~~				al F									
					Met	Leu	Tyr	Thr	Ser	Gln	Leu	Leu	Gly	Leu	Leu
1	AAG	CTT	GCC	GCC	ATG	CTG	TAT	ACC	TCT	CAG	CTG	ATT	GGA	CTA	CTT
								BglI							
										-	HIV	qp12	20 do	main	ì
	Tau	Dha	Trn	Tle	Ser	Ala	Ser						Gly		
A.C	CTC	mmm.	TCC	ATC	TCG	CCT	TCG	ACA	TCT	ATG	СТС	СТТ	GGĞ	ATA	TTG
46	CIG	111	100	AIC	71-	mb	C1.	T	TON	Twn	77-1	Thr	Val	Tur	Tur
0.4	Met	TTE	Cys	ser	Ald	1111	GIU	TIÀD	TITC	TIP	CTC	7 (7)	CTC	ተ እ ተ	ייע די
91	A'I'G	ATC	T.G.T.	AGT	GCT	ACA	GAA	AAA	TIG	TGG	GIC.	ACA	GTC	C	712
	Gly	Val	Pro	Val	Trp	Arg	Glu	Ala	Tnr	Thr	Thr	ьeu	Phe	Cys	Ala
136	GGG	GTA	CCT	GTG	TGG	AGA	GAA	GCA	ACC	ACC	ACT	CTA	TTT	TGT	GCA
	Ser	Asp	Ala	Lys	Ala	Tyr	Asp	Thr	Glu	Val	His	Asn	Val	Trp	Ala
181	TCA	GAT	GCT	AAA	GCC	TAT	GAT	ACA	GAG	GTA	CAT	AAT	GTT	TGG	GCC
	Thr	His	Ala	Cys	Val	Pro	Thr	Asp	Pro	Asn	Pro	Gln	Glu	Val	Val
226	ACA	CAT	GCC	TGT	GTA	CCC	ACA	GAC	CCC	AAC	CCA	CAA	GAA	GTA	GTA
	T.e.11	Glv	Asn	Val	Thr	Glu	Asn	Phe	Asn	Met	Trp	Lys	Asn	Asn	Met
271	TTC	CCA	ΣΔΤ	GTG	ACA	GAA	TAA	ጥጥጥ	AAC	ATG	TGG	AĀA	AAT	AAC	ATG
211	1701	7.00	Cln	Mo+	Uic	Glu	Asn	T1e	Tle	Ser	Len	Tro	Asp	Glu	Ser
216	Vdl	ASP	GIII	7000	CVII	CAC	CV T	אידא	አጥር	ACT	TTZ	TGG	GAT	GAA	AGC
316	GTA	GAT	ÇAG	AIG	CAI	JAG.	JA1	Mp 20	Dmo	TON	Cvc	V-1	Thr	LOU	Aen
	Leu	ьуs	Pro	Cys	vaı	ьуѕ	ьeu	1111	CCD	TEA	Cys	CTT	7 (7	TTTT	7 7 T
361	CTA	AAG	CCA	TGT	GTA	AAA	TTA	ACC	CCA	CTC	IGI	GII	ACT	TIM	AAI
	Cys	Thr	Asn	Leu	Asn	lle	Thr	Lys	Asn	Thr	Thr	ASII	Pro	7 111T	261
406	TGC	ACT	AAT	TTG	AAT	ATC	ACT	AAG	AAT.	ACT	ACT	AAT	CCC	ACT	AGI
	Ser	Ser	Trp	Gly	Met	Met	Glu	Lys	Gly	Glu	Ile	Lys	Asn	Cys	Ser
451	AGC	AGC	TGG	GGA	ATG	ATG	GAG	AAA	GGA	GAA	ATA	AAA	AAT	TGC	TCT
	Phe	Tyr	Ile	Thr	Thr	Ser	Ile	Arg	Asn	Lys	Val	Lys	Lys	Glu	Tyr
496	TTC	TAT	ATC	ACC	ACA	AGC	ATA	AGA	AAT	AAG	GTA	AAG	AAA	GAA	TAT
	Ala	Leu	Phe	Asn	Ara	Leu	Asp	Val	Val	Pro	Ile	Glu	Asn	Thr	Asn
541	GCA	СТТ	ጥጥጥ	AAT	AGA	CTT	GAT	GTA	GTA	CCA	ATA	GAA	AAT	ACT	AAT
011	Asn	Thr	LVS	Tyr	Ara	Leu	Ile	Ser	Cvs	Asn	Thr	Ser	Val	Ile	Thr
586	7 15 II	АСТ	DDC	ТДТ	AGG	TTA	ATA	AGT	тĠт	AAC	ACC	TCA	GTC	ATT	ACA
500	Cla	71.	C11C	Dro	Tare	Val	Ser	Phe	Gln	Pro	Tle	Pro	Ile	His	Tvr
C 2 1	GIII	CCC	C y S	CCA	777	CTA	TCC	TIIC.	CAG	CCA	ΣΤΤ	CCC	ATA	CAT	ΤΆΤ
631	CAG	57-3	IGI	71a	Clar	Dho	710	Mot	Tau	T.378	CAS	Asn	Asn	LVS	Thr
	Cys	vaı	Pro	Ald	GTÀ	PILE	CCC	V m C	CTA	A A C	TCT	7 7 C	7 ነ ነ ጥ	DAG	$\Delta \subset \Delta$
676	TGT	GTC	CCG	GCT	GGG	TIT	GCG	AIG	OIA	. AAG	191	The	AAT	Cln	Cvc
	Phe	Asn	GLy	Ser	GLY	Pro	Cys	Thr	ASII	var	261	1111	Val	GTII	mcm Cys
721	TTC	AAT	GGA	TCA	GGA	CCA	TGC	ACA	AAT	GTC	AGC	ACA	GTA	CAA	161
	Thr	His	Gly	Ile	Arg	Pro	Val	Val	Ser	Thr	GIn	Leu	Leu	Leu	Asn
766	ACA	CAT	GGA	ATT	AGG	CCA	GTG	GTG	TCA	ACT	CAA	CTG	CTG	TTA	AA'I'
	Gly	Ser	Leu	Ala	Glu	Glu	Asp	${ t Ile}$	Val	Ile	Arg	Ser	Glu	Asn	Phe
811	GGC	AGT	CTA	GCA	. GAA	GAA	GAC	ATA	GTA	ATT	AGA	TCT	GAA	AAT	TTC
	Thr	Asp	Asn	Ala	Lys	Thr	Ile	Ile	Val	. Gln	Leu	Asn	Glu	Ser	Val
856	ACA	GAC	AAT	GCT	AAA	ACC	ATA	ATA	GTA	CAG	CTA	AAT	GAA	TCT	GTA
000	Val	Tle	Asn	Cvs	Thr	Ara	Pro	Asn	Asn	Asn	Thr	Arg	Arg	Arg	Leu
901	GTA	ATT	AAT	ТСТ	ACA	AGA	CCC	AAC	AAC	: AAT	ACA	. AGĀ	AGA	AGG	TTA
J O 1	Cor	. Tla	Glv	Pro	Glv	Ara	Δla	Phe	Tvr	· Ala	Ara	Ara	Asn	Ile	Ile
946	DCT.	י אידי	CCA	CCA	CCC	ACA	GCA	ጥጥጥ	רבי	GCA	AGA	AGA	AAC	ATA	ATA
940	C1	. 7 c.	. Tla	7 20	. Gla	Δ1 =	Hie	Cve	Asr	T16	Ser	Ara	Ala	Lvs	Trp
0.04	СТУ	ASP	, 1TE	. AIG	GIII	CCA		THE THE	777	י אייים	NOT	77.27	GCA	777	TGG
991	GGA	GAT	ATA	AGA	CAA	GCA	CAI	161	TIO	, L	TOI	7~~	Clu	TILL	Dha
	Asn	Asn	Thr	Leu	GIN	GIN	TTE	val	. 116	тус	ne.	ALG	CAA	туул	Phe
1036	AAI	' AAC	: ACI	TTA	CAA	CAG	ATA	GTT	ATF	AAA.	TTA	AGA	. GAA	AAA	TTT
	Arg	Asr	ı Lys	Thr	: Ile	Ala	Phe	Asn	Glr	ser -	Ser	. GTĀ	GTĀ	Asp	Pro
1081	AGG	FAA :	' AAA	ACA	ATA	GCC	TTI	' AAT	CAP	A TCC	TCA	GGA	GGG	GAC	CCA
	Glu	ı Ile	val	Met	His	Ser	: Phe	: Asn	Cys	Gly	7 Gly	√ Glu	Phe	Phe	Tyr
1126	GAP	ATT	GTA	ATG	CAC	AGT	TTT	' AAT	TGT	r GG <i>F</i>	GGC	GAA	TTC	TTC	TAC
	Cvs	Asr	Thr	Ala	Glr	Leu	ı Phe	Asr.	Ser	r Thi	Trp	Asr.	ı Val	Thr	Gly
1171	TGT	' AA'	' ACA	A GCF	CAA	CTG	TTT	' AAT	' AG	r aci	TGG	FAA :	GTT	ACT	' GGA
	Glv	7 Thr	Asr	Glv	7 Thr	Glu	ı Gly	Asn	ı Ası	o Ile	: Ile	Thr	Leu	Gln	Cys
	1	· · · -					_		-						

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Figure 3A (continued).

Sequence and translation of two cDNAs encoding HIV gp120-CD154 LONG form extracellular domain fusion proteins.

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1216
      GGG ACA AAT GGC ACT GAA GGA AAT GAC ATA ATC ACA CTC CAA TGC
      Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala
      AGA ATA AAA CAA ATT ATA AAT ATG TGG CAG AAA GTA GGA AAA GCA
1261
      Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn
      ATG TAT GCC CCT CCC ATC ACA GGA CAA ATT AGA TGT TCA TCA AAT
1306
      Ile Thr Gly Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu
      ATT ACA GGG CTG CTA CTA ACA AGA GAT GGA GGT AAT AGT ACT GAG
1351
      Thr Glu Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp
      ACT GAG ACT GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC
1396
      Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu
      1441
      Pro Ile Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Thr Val Gln
      CCA ATA GGA GTA GCA CCC ACC AGG GCA AAG AGA AGA ACA GTG CAA
1486
      Arg Glu Lys Arg
```

1531 AGA GAA AAA AGA

(Gly₄Ser)₃ linker

BamHI

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro 1543 GGG GGA GGC GGT TCA GGA GGT GGA GGT TCT GGA GGT GGC GGA TCG GAT CCA

OR ProAspPro linker

 ${\tt BamHI}$

Pro Asp Pro CC**G GAT CC**A

CD154 LONG FORM Extracellular Domain

1594GS			Leu												
1552PDP			TTG												
1621GS			Leu												
1579PDP			CTT												
1666GS			Thr												
1624PDP			ACA												
1711GS			Ser												
1669PDP			AGC												
1756GS			Glu												
1714PDP			GAG												
1801GS			Asn												
1759PDP			AAT												
1846GS			Thr												
1804PDP			ACA												
1891GS			Ser												
1849PDP	ACC	ATG	AGC	AAC	AAC	TTG	GTA	ACC	CTG	GAA	AAT	GGG	AAA	CAG	CTG
1936GS			Lys												
1894PDP			AAA												
1981GS	Phe	Cys	Ser	Asn	Arg	Glu	Ala	Ser	Ser	Gln	Ala	Pro	Phe	Ile	Ala
1939PDP			TCC												
2026GS			Cys												
1984PDP			TGC												
2071GS			Ala												
2029PDP			GCA												
2116GS			His												
2074PDP			CAC												
2161GS	Val	Phe	Val	Asn	Val	Thr	Asp	Pro	Ser	Gln	Val	Ser	His	Gly	Thr
2119PDP	GTG	TTT	GTC	AAT	GTG	ACT	GAT	CCA	AGC	CAA	GTG	AGC	CAT	GGC	ACT
													Xb	-	
2206GS			Thr												
2164PDP	GGC	TTC	ACG	TCC	TTT	GGC	TTA	CTC	AAA	CTC	GAG	TGA	TAA	TCT	AGA

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Figure 3B.
Sequence and translation of two cDNAs encoding HIV gp120-CD154 short form extracellular domain fusion proteins.

Signal Peptide Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Ger Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu CTT CT CT GT		HindIII														
Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Bq Leu				C .		Sign	nal B	ept:	ide							
Leu Phe Trp Ile Ser Ala Ser Arg Ser Met Leu Leu Gly 11						_		_		Ser	Gln	Leu	Leu	Gly	Leu	Leu
Hard Heave	1	AAG	CTT	GCC	GCC											
Leu Phe Tro IIe Ser Ala Ser Arg Ser Met Leu Leu Gly IIe Leu Met IIe Cys Ser Ala Thr Glu Lys Leu Trp Val Tyr Tyr Tyr Arg Ard Ard Tro Ard Crc Tro God Ard Tro Ard Crc Tro God Ard Tro Ard Gro Crc Tro God Ard Tro Ard Gro Crc Tro God Ard Tro Ard Gro Val Pro Val Trp Arg Glu Val Pro Val Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala God God Gro Ard Tro Ard Gro Crc Tro Ard Gro Val Pro Val Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala God Gro Cra Tro Ard Gro Ard Gro Ard Ard Gro Ard Ard Gro Ard Ard Gro Ard Ard Gro Tra Ard Gro Cra Ard Gro Ard																
Leu Phe Trp Ile Ser Ala Ser Arg Ser Met Leu Leu Leu CTG GTT TGG ATC TCG GCT TCG AGA TCT ATG GCT TCT GGG ATA TTG Met Ile Cys Ser Ala Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr G1 ATG GTC TCT AGG GTA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT G1 GY Val Pro Val Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala 136 GGG GTA CCT GTG TGG AGA GAA AAA TTG TGG GTC ACA GTC TAT TAT TGT GGG GTA ACA GTC GTG TGG AGA GAA GAA CAC ACC ACC ACT CTA TTT TGT GCA Ser Asa Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala 131 TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val Val Cac GCC CC GTG GTA CCC ACA GAC CCA ACC CAC ACA GAA GAA TAG GTA GTA Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asn Phe Asn Met Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Glu Ser Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu Asn 16C ACT AGT AGA GAC CCA CTC TAT ACC ACA AGA CAC CAC CAC CAC CAC CAC CAC									_			HIV	ap12	20 đ	omair	2
Met Ile Cys Ser Ala Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr		Len	Phe	Tro	Tle	Ser	Ala	Ser								
Met Ile Gys Ser Ala Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly ATG ATC TGT AGT GCT ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT Gly Val Pro Val Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala 136 GGG GTA CCT GTG TGG AGA GAA ACA ACC ACC ACT CTA TTT TGT GCA SER Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asa Val Trp Ala 141 And Tr Asp Thr Glu Val His Asa Val Trp Ala 141 And Acc ACT ACC ACT CTA TTT TGG GCA ACC ACC ACC ACC ACC ACC ACC ACC A	46			-					_					_		
91 ATG ATC TGT AGT GCT ACA GAA AAA TTG TGG GTC ACA GTC TĀT TĀT GGY VAI Pro VAI Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala 16 GGG GTA CCT GTG TGG ACA GAA GCA ACC ACC ACT CTA TTT TGT GCA SEG AAP ALA Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala 181 TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC THR His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val Val CLG GTA CAC ACT GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC THR His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val Val CLG GLY ANA GCT GCC TGT GTA CCC ACA GAC CCC ACA GAA GTA GTA GTA CLG GLG GLY ASP VAL THR GLU Asn Phe Asn Met Trp Lys Asn Asn Met CLG GLY Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met CLG GTA GAT GAT GAT ACT GAG GAT ATA ACT ACT GG GAT GAA AAT ACC ACT GAG GAT ATA ACT ACT TTA TGG GAT GAA ACC CAT AAG GAT GAT ATA ACT ACT TTA TGG GAT GAA ACC CAT AAG GAT ATA ACT ACT TTA TGG GAT GAA ACC CAT AAG GAT ATA ACT ACT TTA TGG GAT GAA ACC CAT AAG GAT ATA ACT ACT TTA TGG GAT GAA ACC CAT AAG GAT ATA ACT ACT AAT CCC ACT ACT AAG CCA TGT GTA AAA TTA ACC CAC CCC TCT GTG GTT ACT TTA AAT CYS THR ASN Leu ASN ILE THR Lys ASN THR THR ASN PRO THR SET SET SET TTP GLY Met Met Glu Lys Gly Glu Ile Lys Asn Cys Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys Ser 451 AGC AGC ACA ACC ATA AGC AAT AGT ACT AAT AAA AAT ACT ACT AAA AAT ACT AC	2 0															
G1y Val Pro Val Trp Arg Glu Ala Thr Thr Leu Phe Cys Ala GG GG GTA CCT GTG AGA GAA GCA ACC ACC ACT CTA TTT TGT GCA Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala 181 TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCA TH His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val Cal Leu Gly Asn Val Thr Glu Sen Phe Asn Met Trp Lys Asn Asn Met Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met Carl GAG ACA CAC CAC CAC GAA GAC CCA CAC CAC	9.1			-					-		-				_	-
136 GGG GTA CCT GTG TGG AGA GAA GCA ACC ACT CTA TTT TGT GCA Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala 181 TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC THR His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Trp Glu Asn Pro GLN GLU Val Val Leu Gly Asn Val Trp Glu Asn Pro GLN GAA GAA GTA GTA CCC ACA CCA CAC CAA GAC CCA GAC CCA GAC CAA GAC GAC	21															
Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala 181 TrQ AGA GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC Thr His Ala Cys Val Fro Thr Asp Fro Asn Fro Gln Glu Val Val 226 ACA CAT GCC TGT GTA CCC ACA GAC CCC ACA CAC GAG GAC CCA CAA GAA G	126															
181	720															
The His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val	101															
226	TRI															
Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met 271 TTG GGA AAT GTG ACA GAA AAT TTT AAC ATG TGG AAA AAT AAC ATG VAL Asp Glu Ser 316 GTA GAT CAG ACA GAT VAL ASP GLU Ser 316 GTA GAT CAG ACA GAT VAL ASP GLU Ser 316 GTA GAT CAG ACA GAT VAL ASP GLU Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu Asn AGA CAT Cys Thr Asn CCA TGT GTA AAA TATA ACC CAC CTC TGT GTT ACT TTA AAC Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro Thr Ser ACC ACT AGT GAT AAT TGA ACC CCA CTC TGT GTT ACT TATA ACC Cys Thr Asn Leu Asn ATC ACT AAG AAT ACT ACT AAT CCC ACT AGT AGT AGC ACC ACT AGT GAG AAA GAA AAT ACT ACT AAAT CCC ACT AGT AGT AGC ACC ACC TGG GGA ATG ATG AGG GAA AAA AAA AACT ACT AAAT CCC ACT AGT AGC ACC ACC ACC ACC ACC ACC ACC ACC ACC	006				_				_							
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Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys Ser		Cys	\mathtt{Thr}	Asn	Leu	Asn	Ile	Thr	Lys	Asn	Thr	Thr	Asn	Pro	Thr	Ser
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Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr		Ser	Ser	Trp	Gly	Met	Met	Glu	Lys	Gly	Glu	Ile	Lys	Asn	Cys	Ser
496 TTC TAT ATC ACC ACA AGC ATA AGA AAT AĀG GTA ĀĀG ĀĀA GĀA TĀT ĀĀG ĀĀA LEU Phe ASN ATG LEU ASP VAL VAL PRO ILE GLU ĀSN THR ASN ASN THR LYS TYR ARG LEU LEU SCR CYS ASN THR SCR VAL LILE THR ASN ASN ASN THR LYS TYR ARG LEU LLE SCR CYS ASN THR SCR VAL LILE THR ASN ASN ACT AAT AGA ATT AGA GTA AGT TAT AGA GTA AGT TAT AGA GGN ALA CCT AAT AGA GLN ALA CCT AAT AGA GTA AGT TAT AGA GGN ALA CCT CA AGG GCC TGT CCA AAG GTA TCC TTT CAG CCA ATT CCC ATA CAT TAT CYS VAL PRO ALG GLY PHO ALA MET LEU LYS CYS ASN THR SCR VAL LYS THR CYS VAL PRO ALG GTY TTG GGA ATG CTA AAG TGT AACA AGA AAT AAG ACA PHO ASN GLY SCR GLY PRO CYS THR ASN VAL SCR THR VAL GLN CYS THR HIS GLY PRO CYS THR ASN VAL SCR THR VAL GLN CYS THR HIS GLY LILE AGG CCA ATT GCC ACA AGT CAA TGT THR HIS GLY LILE AGG CCA GTG GTG TCA ACT CAA GTG TA AAG TGA ACA CAT GGA ATT AGG CCA GTG GTG TCA ACT CAA CTG CTG TTA AAT GLY SCR LILE AGG CAG GTG GTG TCA ACT CAA CTG CTG TTA AAT GLY SCR LILE AGG CAG GTG GTG TCA ACT CAA CTG CTG TTA AAT GLY SCR LILE AGG CAG GTG GTG TCA ACT CAA CTG CTG TTA AAT TTC THR ASP ASN ALA LYS THR LILE VAL LEU LEU LEU LEU LEU LEU LEU LEU LEU LE	451	AGC	AGC	TGG	GGA	ATG	ATG	GAG	AAA	GGA	GAA	ATA	AAA	AAT	TGC	TCT
Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn GCA CTT TTT AAT AGA CTT GAT GTA GTA CCA ATA GAA AAT ACT AAT ASN Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Ser CAC GTA GTA GTA GTA GTA GTA GTA ACT ATT AGA AAT ACT ATA AGT TGT AAC ACC TCA GTC ATT ACA GIN Ala Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr GTA		Phe	Tyr	Ile	Thr	Thr	Ser	Ile	Arg	Asn	Lys	Val	Lys	Lys	Glu	Tyr
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676 Cys Val Pro Ala Gly Phe Ala Met Leu Lys Cys Asn Lys Thr 676 TGT GTC CCG GCT GGG TTT GCG ATG CTA AAG TGT AAC AAT AAC AAT AAG ACA ACA AAG GTG ACA AAG ACA ACA GTG CAA ATG ACA AAT AGG CCA GTG GTC ACA ACA CAC CTG ACA ACA CAC ACA		Gln	Ala	Cys	Pro	Lys	Val	Ser	Phe	Gln	Pro	Ile	Pro	Ile	His	Tyr
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Phe Asn Gly Ser Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys TTC AAT GGA TCA GGA CCA TGC ACA AAT GTC AGC ACA GTA CAA TGT Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn ACA CAT GGA ATT AGG CCA GTG GTG TCA ACT CAA CTG CTG TTA AAT Gly Ser Leu Ala Glu Glu Asp Ile Val Ile Arg Ser Glu Asn Phe B11 GGC AGT CTA GCA GAA GAA GAC ATA GTA ATT AGA TCT GAA AAT TTC Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Glu Ser Val ACA GAC AAT GCT AAA ACC ATA ATA GTA CAG CTA AAT GAA TCT GTA Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu B11 GTA ATT AAT TGT ACA AGA CCC AAC AAC AAC AAT ACA AGA AGA AGG TTA Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile B12 GGA GAT ATA AGA CCA GGA GCA TTT TAT GCA AGA AGA AGA ACA ATA ATA GLY Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp B13 GGA GAT ATA AAC ACA CAA GCA CAT TGT AAC ATT AGA GGA GCA AAA TGG Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu Lys Phe AAA AAC ACT TTA CAA CAG ATA GTT ATA AAA TTA AGA GAA AAA TTT AGG AAT AAC ACT TTA CAA CAG ATA GTT ATA AAA TTA AGA GAG GGG GAC CCA GGU Ile Val Met His Ser Phe Asn Gln Ser Ser Gly Gly Asp Pro AGG AAT AAC ATT GTA ATG CAC CAT TTT AAT CAA TCC TCA GGA GGG GAC CCA GGU Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr AGG AAA TTC TTC TAC Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr Gly	676	-				_									_	
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7/7 Figure 3B (Continued). Sequence and translation of two cDNAs encoding HIV gp120-CD154 short form extracellular domain fusion proteins.

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XbaI

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2068GS Arg 2026PDP AGA a valid OMB control number.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

■ Declaration OR Submitted with Initial Filing

Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e)) required)

Attorney Docket Num	ber						
First Named Inventor		Jeffrey Ledbetter					
COMPLE	TE II	KNOWN					
Application Number		/					
Filing Date	•						
Group Art Unit							
Examiner Name							

As a below named inventor, I hereby declare that:									
My residence, post office address,	and citizenship are	as stated below next to my	name.						
I believe I am the original, first and names are listed below) of the sub	I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:								
DNA Vaccines Encoding Antigen Linked to a Domain That Binds CD40.									
the specification of which (Title of the Invention)									
is attached hereto OR									
was filed on (MM/DD/YYYY) as United States Application Number or PCT International									
Application Number	Application Number and was amended on (MM/DD/YYYY) (if applicable).								
I hereby state that I have reviewed	and understand the	contents of the above ider	itified specificatio	n, including the claims	, as				
amended by any amendment specifically referred to above.									
I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.									
I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.									
Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy A	ttached?				
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Additional foreign application nui	nbers are listed on a	supplemental priority data	a sheet PTO/SB/	2B attached hereto:					
I hereby claim the benefit under 35									
Application Number(s)	Filing Date	e (MM/DD/YYYY)							
		_		onal provisional app ers are listed on a	plication				
US60/159,690	10/14/9	99		emental priority data	a sheet				
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[Page 1 of 2]
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DECLARATION — Utility or Design Patent Application

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DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet Page ___ of ___

		_								
Name of Addition	A petition has been filed for this unsigned inventor									
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Mart	ha.			ŀ	łayde	n-Ledbet	ter			
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City	Shoreline	State	WA		ZIP	98177	Count	ry U	SA.	
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Given Name (first and middle [if any]) Family Name or							me or	Sumame		
Inventor's Signature									ate	
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Given Na	me (first and middle [if any)				Family Na	me or	Sumam	е	
Inventor's Signature									ate	
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      binds CD40
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       binds CD40
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long form from amino acids 48 (Arg) to 261 (Leu)+Glu

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Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu 35 40 45

Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr 50 60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro 65 70 75 80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met 85 90 95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu 100 105 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val 115 120 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro 130 135 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys 145 150 155 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr \$165\$ \$170\$ \$175\$

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn 180 185 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala 195 200 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro 210 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser 225 230 235

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
245 250 255

Pro Val Val Ser Thr Gln Leu Leu Asn Gly Ser Leu Ala Glu Glu 260 265 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile 280 Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn 295 Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr 455 Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys 570 Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser 600 Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly 615

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Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
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Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
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Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
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Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr 50 60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro

65					70					75					80
Asn	Pro	Gln	Glu	Val 85	Val	Leu	Gly	Asn	Val 90	Thr	Glu	Asn	Phe	Asn 95	Met
Trp	Lys	Asn	Asn 100	Met	Val	Asp	Gln	Met 105	His	Glu	Asp	Ile	Ile 110	Ser	Leu
Trp	Asp	G1u 115	Ser	Leu	Lys	Pro	Cys 120	Val	Lys	Leu	Thr	Pro 125	Leu	Cys	Val
Thr	Leu 130	Asn	Cys	Thr	Asn	Leu 135	Asn	Ile	Thr	Lys	Asn 140	Thr	Thr	Asn	Pro
Thr 145	Ser	Ser	Ser	Trp	Gly 150	Met	Met	Glu	Lys	Gly 155	Glu	Ile	Lys	Asn	Cys 160
Ser	Phe	Tyr	Ile	Thr 165	Thr	Ser	Ile	Arg	Asn 170	Lys	Val	Lys	Lys	Glu 175	Tyr
Ala	Leu	Phe	Asn 180	Arg	Leu	Asp	Val	Val 185	Pro	Ile	Glu	Asn	Thr 190	Asn	Asn
Thr	Lys	Tyr 195	Arg	Leu	Ile	Ser	Cys 200	Asn	Thr	Ser	Val	Ile 205	Thr	Gln	Ala
Cys	Pro 210	Lys	Val	Ser	Phe	Gln 2 1 5	Pro	Ile	Pro	Ile	His 220	Tyr	Cys	Val	Pro
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Gly	Pro	Cys	Thr	Asn 245	Val	Ser	Thr	Val	Gln 250	Cys	Thr	His	Gly	Ile 255	Arg
Pro	Val	Val	Ser 260	Thr	Gln	Leu	Leu	Leu 265	Asn	G1y	Ser	Leu	Ala 270	Glu	Glu
Asp	Ile	Val 275	Ile	Arg	Ser	Glu	Asn 280	Phe	Thr	Asp	Asn	Ala 285	Lys	Thr	Ile
Ile	Val 290	Gln	Leu	Asn	Glu	Ser 295	Val	Val	Ile	Asn	Cys 300	Thr	Arg	Pro	Asn
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Ala	Arg	Arg	Asn	Ile 325	Ile	Gly	Asp	Ile	Arg 330	Gln	Ala	His	Cys	Asn 335	Ile
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Arg	Glu	Lys 355	Phe	Arg	Asn	Lys	Thr 360	Ile	Ala	Phe	Asn	Gln 365	Ser	Ser	Gly
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Phe 385	Tyr	Cys	Asn	Thr	Ala 390	Gln	Leu	Phe	Asn	Ser 395	Thr	Trp	Asn	Val	Thr 400
Gly	Gly	Thr	Asn	Gly 405	Thr	Glu	Gly	Asn	Asp 410	Ile	Ile	Thr	Leu	Gln 415	Cys

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Pro Asp Pro Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp 515 520 Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly 615 Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe 695 Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu 725

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<222>
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<223> HIV gp120 domain plus (gly4ser)3 linker
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      (528)..(682)
      CD154 extracellular domain
       short form from amino acids 108 (Glu) to 261 (Leu)+Glu
       Binds CD40
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Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
Ala Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr 50 60
Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro 65 70 75 80
Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
    130
                        135
Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
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225		230				235					240
Gly Pro Cys	Thr Asn 245	Val Se	r Thr	Val	Gln 250	Суѕ	Thr	His	Gly	Ile 255	Arg
Pro Val Val	Ser Thr 260	Gln Le	u Leu	Leu 265	Asn	Gly	Ser	Leu	Ala 270	Glu	Glu
Asp Ile Val 275	_	Ser Gl	u Asn 280		Thr	Asp	Asn	Ala 285	Lys	Thr	Ile
Ile Val Gln 290	Leu Asn	Glu Se 29		Val	Ile	Asn	Cys 300	Thr	Arg	Pro	Asn
Asn Asn Thr 305	Arg Arg	Arg Le 310	u Ser	Ile	Gly	Pro 315	Gly	Arg	Ala	Phe	Tyr 320
Ala Arg Arg	Asn Ile 325	Ile Gl	y Asp	Ile	Arg 330	Gln	Ala	His	Cys	Asn 335	Ile
Ser Arg Ala	Lys Trp 340	Asn As	n Thr	Leu 345	Gln	Gln	Ile	Val	Ile 350	Lys	Leu
Arg Glu Lys 355		Asn Ly	s Thr 360	Ile	Ala	Phe	Asn	Gln 365	Ser	Ser	Gly
Gly Asp Pro 370	Glu Ile	Val Me 37		Ser	Phe	Asn	380	Gly	Gly	Glu	Phe
Phe Tyr Cys 385	Asn Thr	Ala Gl 390	n Leu	Phe	Asn	Ser 395	Thr	Trp	Asn	Val	Thr 400
Gly Gly Thr	Asn Gly 405	Thr Gl	u Gly	Asn	Asp 410	Ile	Ile	Thr	Leu	Gln 415	Cys
Arg Ile Lys	Gln Ile 420	Ile As	n Met	Trp 425	Gln	Lys	Val	Gly	Lys 430	Ala	Met
Tyr Ala Pro 435		Thr Gl	y Gln 440	Ile	Arg	Cys	Ser	Ser 445	Asn	Ile	Thr
Gly Leu Leu 450	Leu Thr	Arg As 45		Gly	Asn	Ser	Thr 460	Glu	Thr	Glu	Thr
Glu Ile Phe 465	Arg Pro	Gly Gl 470	y Gly	Asp	Met	Arg 475	Asp	Asn	Trp	Arg	Ser 480
Glu Leu Tyr	Lys Tyr 485	Lys Va	l Val	Arg	Ile 490	Glu	Pro	Ile	Gly	Val 495	Ala
Pro Thr Arg	Ala Lys 500	Arg Ar	g Thr	Val 505	Gln	Arg	Glu	Lys	Arg 510	Gly	Gly
Gly Gly Ser 515	Gly Gly	Gly Gl	y Ser 520	Gly	Gly	Gly	Gly	Ser 525	Asp	Pro	Glu
Asn Ser Phe 530	Glu Met	Gln Ly 53		Asp	Gln	Asn	Pro 540	Gln	Ile	Ala	Ala
His Val Ile 545	Ser Glu	Ala Se 550	r Ser	Lys	Thr	Thr 555	Ser	Val	Leu	Gln	Trp 560
Ala Glu Lys	Gly Tyr 565	Tyr Th	r Met	Ser	Asn 570	Asn	Leu	Val	Thr	Leu 575	Glu

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Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr 580 585 590
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- Ala Gl
n Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gl
n Ala Pro $595 \hspace{1.5cm} 600 \hspace{1.5cm} 605$
- Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile 610 615 620
- Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln 625 630 635
- Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser 645 650 655
- Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly
 660 665 670
- Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu 675 680
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- <211> 668
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- <223> Synthetic secretory signal peptide
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- <222> (21)..(513)
- <223> HIV gp120 domain with ProAspPro linker
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- <222> (514)..(668)
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- Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser 1 $$ 5 $$ 10 $$ 15
- Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr 20 25 30
- Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu 35 40 45
- Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr 50 55 60
- Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro 65 70 75 80
- Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met 85 90 95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro 135 Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr 165 Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser 230 Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu 260 265 Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile 280 Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn 295 Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile 325 330 Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly 360 Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr 390 Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys 410 Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr

	435					440					445			
Gly Le 45		Leu	Thr	Arg	Asp 455	Gly	Gly	Asn	Ser	Thr 460	Glu	Thr	Glu	Thr
Glu Il 465	e Phe	Arg	Pro	Gly 470	Gly	Gly	Asp	Met	Arg 475	Asp	Asn	Trp	Arg	Ser 480
Glu Le	u Tyr	Lys	Tyr 485	Lys	Val	Val	Arg	Ile 490	Glu	Pro	Ile	Gly	Val 495	Ala
Pro Th	r Arg	Ala 500	Lys	Arg	Arg	Thr	Val 505	Gln	Arg	Glu	Lys	Arg 510	Pro	Asp
Pro Gl	u Asn 515	Ser	Phe	Glu	Met	Gln 520	Lys	Gly	Asp	Gln	Asn 525	Pro	Gln	Ile
Ala Al 53		Val	Ile	Ser	Glu 535	Ala	Ser	Ser	Lys	Thr 540	Thr	Ser	Val	Leu
Gln Tr 545	p Ala	Glu	Lys	Gly 550	Tyr	Tyr	Thr	Met	Ser 555	Asn	Asn	Leu	Val	Thr 560
Leu Gl	u Asn	Gly	Lys 565	Gln	Leu	Thr	Val	Lys 570	Arg	Gln	Gly	Leu	Tyr 575	Tyr
Ile Ty	r Ala	Gln 580	Val	Thr	Phe	Суѕ	Ser 585	Asn	Arg	Glu	Ala	Ser 590	Ser	Gln
Ala Pr	o Phe 595	Ile	Ala	Ser	Leu	Cys 600	Leu	Lys	Ser	Pro	Gly 605	Arg	Phe	Glu
Arg Il 61		Leu	Arg	Ala	Ala 615	Asn	Thr	His	Ser	Ser 620	Ala	Lys	Pro	Cys
Gly Gl 625	n Gln	Ser	Ile	His 630	Leu	Gly	Gly	Val	Phe 635	Glu	Leu	Gln	Pro	Gly 640
Ala Se	er Val	Phe	Val 645	Asn	Val	Thr	Asp	Pro 650	Ser	Gln	Val	Ser	His 655	Gly
Thr Gl	y Phe	Thr 660	Ser	Phe	Gly	Leu	Leu 665	Lys	Leu	Glu				
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<222>														
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<220> <221> <222> <223>		(2		ellu	lar	doma	in							

long form from amino acids 48 (Arg) to 261 (Leu)+Glu binds CD40 $\,$

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Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser 1 5 10 15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr 20 25 30

Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg 35 40 45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Gly 50 55 60

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Arg 65 70 75 80

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
85 90 95

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser 100 105 110

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys 115 120 125

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu 130 135 140

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser 145 150 155 160

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly 165 170 175

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
180 185 190

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr $195 \hspace{1.5cm} 200 \hspace{1.5cm} 205 \hspace{1.5cm}$

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser 210 215 220

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala 225 230 235 240

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His 245 250 255

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn 265 270

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe 275 280 285

Gly Leu Leu Lys Leu Glu 290

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<220>
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       BINDING
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       CD154 extracellular domain
       long form from amino acids 48 (Arg) to 261 (Leu)+Glu
       binds CD40
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Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Pro Asp
Pro Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp
Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser
Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe
                                105
Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser
Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val
                        135
Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu
Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly
Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
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Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu

210 215 220

Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser

Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe 245

Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr 265

Ser Phe Gly Leu Leu Lys Leu Glu

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<211> 234 <212> PRT

<213> HIV-HUMAN FUSION PROTEIN

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<222> (21)..(77)

<223> HIV gp120 V3 loop plus (gly4ser)3 linker

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<222> (80)..(234)

<223> CD154 extracellular domain short form from amino acids 108 (Glu) to 261 (Leu)+Glu binds CD40

<400> 26

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Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Gly

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Glu

Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala

His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp

Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu 120

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Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr
Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro
                                        155
Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln
            180
Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser
                            200
Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly
Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
                    230
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       Binds CD40
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Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Pro Asp
Pro Glu Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile
                    70
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Ala Ala His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu

- Gln Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr $100 \hspace{1.5cm} 105 \hspace{1.5cm} 110$
- Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr 115 120 125
- Ile Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln 130 140
- Ala Pro Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu 145 150 155 160
- Arg Ile Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys \$165\$ \$170\$ \$175\$
- Gly Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly 180 185
- Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly 195 200 205
- Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu 210 215 220